STUDIES OF THE SOLUBILITY OF THE PRODUCT OF ARGENTAFFIN AND ARGYROPHIL REACTIONS

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The reaction of silver ion with a tissue usually is considered to be due to the presence of an exogenous or endogenous reducing substance or group of substances. When the reducing chemical substance is attached to the tissue itself, the blackening of this substance by the silver metal has been termed an "argentaffin" reaction. When the reducing substance must be supplied to the environment in which the reaction is occurring, the term "argyrophil reaction" has been used. Ascorbic acid, phenolic compounds, aldehydes, and uric acid are substances contained in tissue which may reduce silver solutions under specific conditions. Melanin in the skin is also an argentaffin substance.

Lillie (4) has shown that there are various types of argentaffin reacting materials. The type of fixation used for the tissue is important because formaldehyde needs to be present in order to produce some of the reactions. The enterochromaffin granules of the Kultschitzky cells give an argentaffin reaction after formalin fixation, and Pearse noted that the reaction is dependent on formaldehyde.

Lillie (5) has demonstrated in the test tube that many compounds of a reducing nature are capable of producing metallic silver from silver nitrate, ammoniacal silver nitrate, and methenamine silver. Substances which reduce silver nitrate at pH 4 were generally the most active. Lillie also has recorded that the use of distilled water at 60° C. will render many argentaffin-producing substances nonreactive, and at times will reverse the argentaffin reaction created by the oxidation of periodic acid. He concluded that the argentaffin reaction appears to be nonspecific and does not necessarily denote the presence of aldehyde groups.

A recent study concerning the histochemical aspects of an argyrophil silver method of staining (8) disclosed that it was formaldehyde dependent. We have found that the argyrophil histologic reaction could be produced without the presence of a reducing substance, only a controllable source of base being necessary. We found too that solubility of the product of the argyrophil reaction in the nerve axoplasm was the same as solubility of silver oxide. These findings have led us to study the solubility of the products of the classic argentaffin reaction.

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METHODS

The methenamine silver method of Gomori and the diammine silver technic of Lillie (3) were used to produce classic examples of the argentaffin reaction. They were used on frozen sections of human ileum, some of which had undergone previous alcoholic extraction for 24 hours, and on paraffin sections of human ileum to demonstrate enterochromaffin granules. The diammine silver technic of Lillie also was used on normally pigmented human skin, pigmented nevi, and blue nevi. The silver nitrate stain of Becker was used for demonstration of melanin in human skin because of its frequent application in the past in standard demonstrations of melanin in the skin. It is considered an argentaffin stain. The argyrophil reaction used in all studies was the frozen-section silver method for axoplasm detailed elsewhere (7). Once the products of the reaction had been formed, they were exposed to the solvents listed in table 1. Untreated sections were used as a control for the color of the tissue component studied.

RESULTS

The frozen-section silver method for axoplasm stained the enterochromaffin granules in the ileum in the same manner as the classic argentaffin reactions did. This argyrophil reaction occurred in untreated frozen sections, frozen sections pretreated with alcohol for 24 hours and sections of tissue embedded in paraffin by a routine method. The product of this reaction was soluble when exposed to 3 per cent solution of hydrogen peroxide for 5 minutes. As may be seen in table 1, the diammine silver and the methenamine silver methods gave positive results

TABLE 1

Silver reactions on enterochromaffin granules and solubility of the product

	Tissue Section Studied*			Solubility of stain
	Frozen	Frozen, cleared with alcohol	Paraffin	
Frozen-section silver for axoplasm (argyrophil)	+	+	+	Removed in 5 minutes by 3 per cent H_2O_2
Diammine silver (argentaffin)	+	0	0	Removed in 5 minutes from frozen sections by 3 per cent H_2O_2
Methenamine silver (argentaffin)	+	0	0	Removed in 5 minutes from frozen sections by 3 per cent H_2O_2

* + = positive
0 = negative

only with the frozen sections of human ileum. When frozen sections were cleared in alcohol, and then returned to water and stained, no argentaffin product resulted. Similarly, no argentaffin reaction was evident from the use of either method in paraffin sections. The positive results obtained with both the diammine silver and the methenamine silver reaction in the frozen sections could be abolished by exposure for 5 minutes to 3 per cent hydrogen peroxide.

Another classic argentaffin substance, melanin, was stained by three methods: the frozen-section silver method for axoplasm, the diammine silver method, and the Becker silver nitrate technic (table 2). In each instance, a blank control was used to establish the color of the melanin in the unstained tissue. The melanin reacted positively to use of the frozen-section silver method for staining axoplasm. Paraffin-fixed sections containing melanin also reacted positively to this method. The product of the reaction was removed by 5 minutes' exposure in 3 per cent hydrogen peroxide (fig. 1*a* and *b*). The melanin was bleached to a light yellow on exposure to the same solution for 48 hours. The product of the argyrophil reaction also was removed by exposure to saturated potassium iodide, sodium thiosulfate, ammonium carbonate, and 28 per cent ammonia in water for 24 hours. The diammine silver reaction occurred in both frozen and paraffin sections. The solubility of the product of the reaction was the same as that shown by the frozen-section argyrophil technic (fig. 2*a* and *b*). Five minutes' exposure of the stained paraffin sections to hydrogen peroxide gave partial, but definite, removal of the stain. When paraffin sections were stained by the Becker silver nitrate technic, the reaction product had the same solubility as the reaction products of the other two methods (fig. 3*a* and *b*).

Application of the frozen-section silver technic for axoplasm, the diammine silver technic, or the Becker silver nitrate technic always caused a marked reaction in tissue that contained a blue nevus. The marked increase in dermal melanin in this state provided an available tissue in

TABLE 2

Silver reactions on melanin granules and the solubility of the product

	Tissue Section Studied*		Solubility of Stain
	Frozen, cleared with alcohol	Paraffin	
Frozen-section silver for axoplasm (argyrophil)	+	+	In all tissue the product was removed from melanin by exposure for 5 minutes to 3 per cent H_2O_2 and by exposure for 24 hours to saturated K I, saturated $Na_2S_2O_3$, saturated $(NH_4)_2\ CO_3$, and 28 per cent NH_3 in water
Diammine silver (argentaffin)	+	+	
Becker's silver nitrate (argentaffin)	—	+	

* + = positive
— = not done

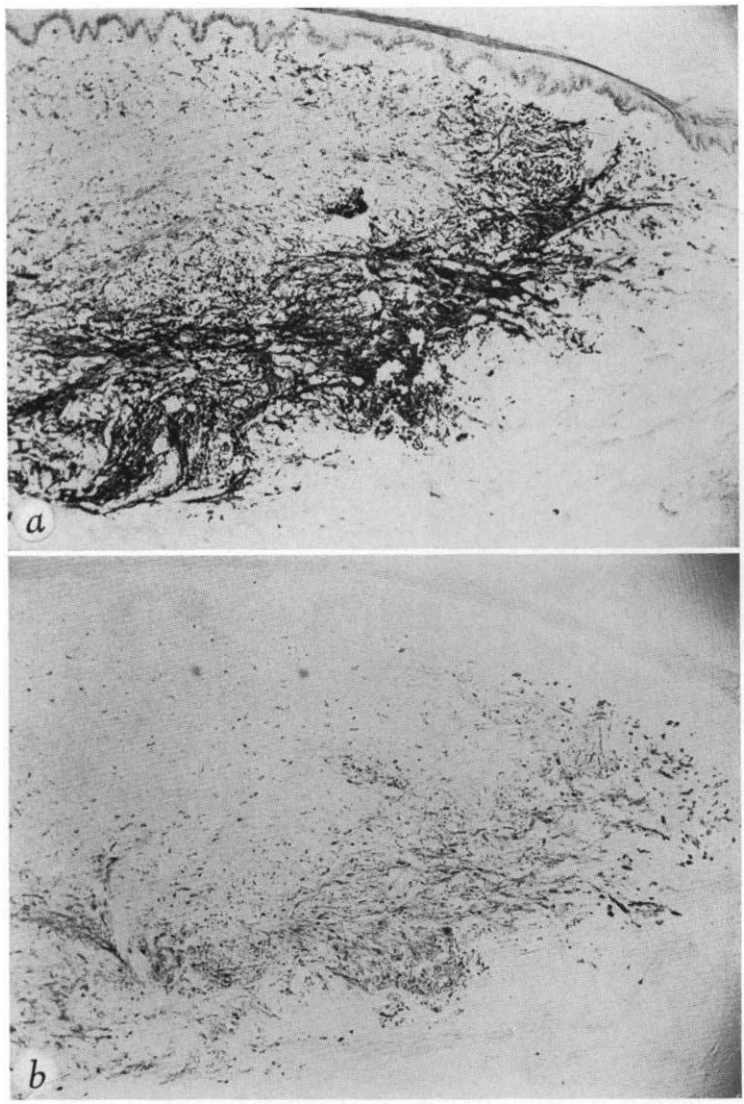

FIG. 1. Blue nevus. *a*. Frozen-section silver method for staining axoplasm has produced an argyrophil reaction in the melanin of the skin (×50). *b*. The product of the argyrophil reaction shown in *a* has been dissolved to a large extent by exposure of the tissue to 3 per cent solution of hydrogen peroxide for 5 minutes (×50).

which to determine the solubility of the product of the reaction. In each instance, the results were qualitatively the same as those for pigmented human epidermis but were quantitatively much more definite.

COMMENT

The argyrophil silver technic used in this study for staining nerve axoplasm produces a product in tissue that usually is considered to be argentaffin in nature. This technic will stain enterochromaffin granules, melanin granules, and nerve axoplasm. In unpublished studies we have shown that chromaffin paraganglia tissue also will stain with this technic. The product of this argyrophil reaction in these separate tissue components is soluble under circumstances considered compatible with the solubility of silver oxide. The classic argentaffin reactions studied produced products in argentaffin sites which

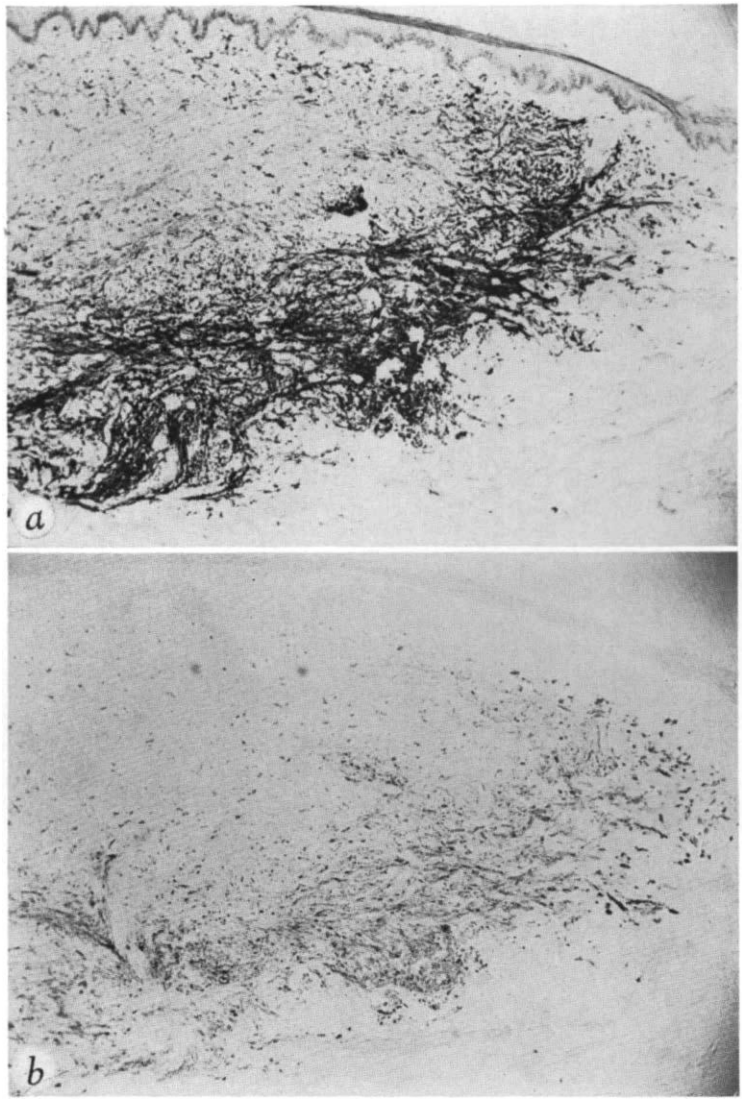

FIG. 2. Blue nevus. *a*. The diammine silver method of Lillie has produced an argentaffin reaction (×50). *b*. A high degree of solubility of the product of the reaction shown in *a* occurred on exposure of the tissue to a saturated solution of potassium iodide for 24 hours (×50).

were similarly soluble. The similarity in solubility of the products of these reactions is remarkable.

One difference apparent between the product of the argentaffin reaction for enterochromaffin granules and the product of the argyrophil reaction in the same substrate is the effect of alcohol. Alcohol prevented methenamine silver and diammine silver from reacting, but not the argyrophil silver. Whether the substrate was altered by alcohol is not known. Yet the product produced in the enterochromaffin granules by classic argentaffin stains displayed the same solubility as that produced by the argyrophil reaction.

It should be pointed out that the argyrophil reaction for nerve axoplasm is dependent on formaldehyde. Similarly the argentaffin reaction of the enterochromaffin granules requires fixation in formaldehyde. No such dependency has

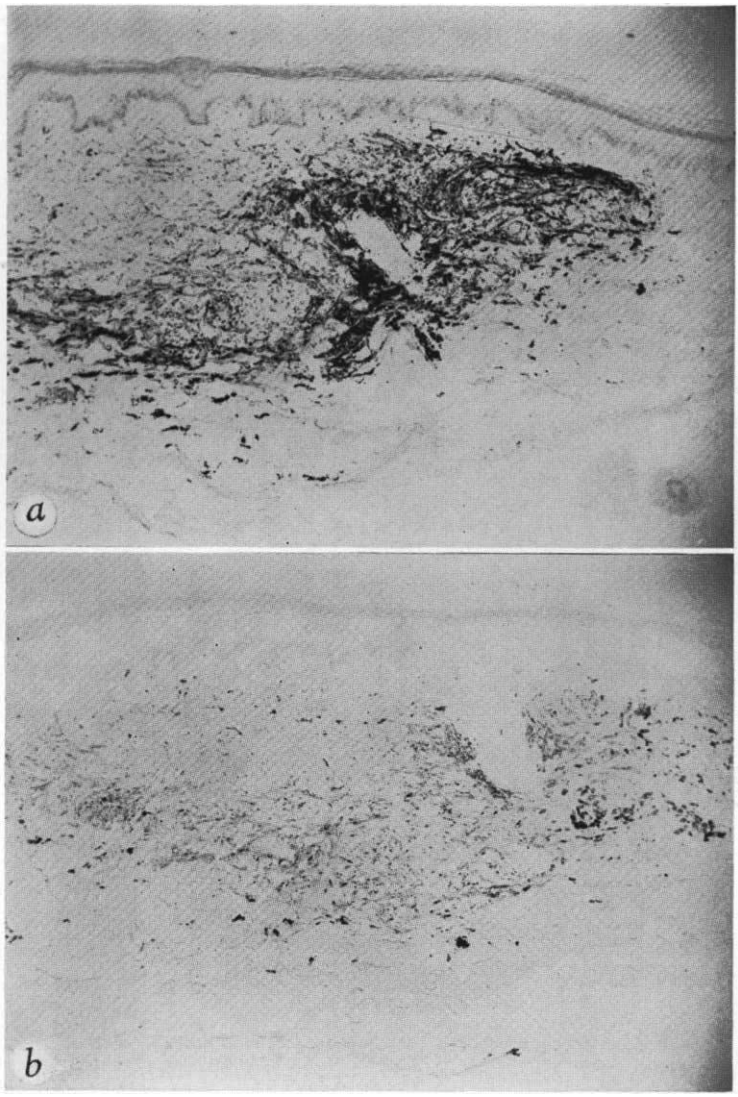

FIG. 3. Blue nevus. *a*. The Becker silver nitrate method has been used to produce an argentaffin product (×50). *b*. A high degree of solubility of the argentaffin product of the Becker silver nitrate method occurred on exposure to a saturated solution of sodium thiosulfate for 24 hours (×50).

been described for melanin in the skin. This appears to be a valid difference between the reactions described.

The similar solubility of the products merits explanation. One explanation could be that all these separate reactions have a final, common chemical pathway. Since silver oxide appears to be the product in each instance, reduction of silver by argentaffin substrate seems doubtful when alkali is present as it is when the diammine and methenamine silver methods are employed. In the simple reaction between the melanin in fresh tissue and silver nitrate, the occurrence of true reduction can be presumed. Yet, even here the solubility of the product is not that of finely divided silver, but rather that of an oxidized form. Further intensive investigation into the mechanism of these reactions is indicated.

SUMMARY

Solubility studies on the products of standard argyrophil reactions for nerves and argentaffin reactions for enterochromaffin granules of human ileum and melanin of normal and diseased skin have given evidence that the products of both types of reaction are identical. The solubility of these products resembles that of silver oxide.

Alcohol prevents the staining of enterochromaffin granules from argentaffin reactions but not from an argyrophil reaction. Alcohol has no effect on the argentaffin reaction of melanin in the skin and the blue nevus.

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